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Molenaar, I; Hulstaert, CE; Vos, J; Hommes, FA

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### Membrane characteristics in vitamin E deficiency and the assessment of vitamin E status

By I. MOLENAAR, C. E. HULSTAERT and J. VOS, *Centre for Medical Electronmicroscopy, University of Groningen, Oostersingel 69/2, Groningen, The Netherlands* and F. A. HOMMES, *Department of Pediatrics, University of Groningen, Groningen, The Netherlands*

After fifty years of research the biological function of vitamin E in cellular metabolism is still not completely understood. The study of this vitamin is made complex by the polymorphous macroscopical and microscopical findings in different mammals and birds with experimental vitamin E deficiency (Scott, 1970) and also by the often extremely diverse methodological approaches to the problem.

However, it is this very situation which could lead to solving the problem from the view point of cell biology. Cells can only organize their cytoplasmic space in the presence of cellular membranes which not only surround but also compartmentalize the cell. The chemical anatomy of the membrane is described in 'membrane models', the most recent being that of Singer & Nicolson (1972). This model describes the membrane as a two-dimensionally oriented solution of globular proteins in a viscous phospholipid bilayer solvent. Membrane properties define to a large extent cellular and tissue properties. Changes in membrane properties introduce functional and morphological aberrations, depending in character upon the kind of membrane affected.

The evidence that vitamin E exerts its biological function in cellular membranes is accumulating. Vitamin E is present in the inner mitochondrial membrane (Oliveira, Weglicki, Nason & Nair, 1969) and in the plasma membrane of erythrocytes (Silber, Winter & Kayden, 1969). Also it was found that membrane-bound vitamin E is functional in certain enzymic reactions during which it is degraded (McCay, Poyer, Pfeifer, May & Gillian, 1971; McCay, Pfeifer & Stipe, 1972). Also there is morphological evidence for this finding (see below).

The theories on vitamin E function, in relation to cellular membranes, have been summarized by Molenaar, Vos & Hommes (1972) as follows: vitamin E, if present in a given membrane, is probably located within it as a complex with the polyunsaturated fatty acids of the phospholipids (Lucy, 1972; Diplock & Lucy, 1973). This location is especially suited for one function of the  $\alpha$ -tocopherol molecule, inhibiting the peroxidation of membrane-bound polyunsaturated fatty acids during electron transport functions (McCay *et al.* 1971; McCay *et al.* 1972). Protection by vitamin E against peroxidation, according to the antioxidant theory of Tappel

(1962), is only found in adipose tissue, where fat is stored as large droplets in the vacuoles of fat cells.

During the last five years, our group has intensively studied the effect of vitamin E deficiency on the ultrastructure and chemical composition of cellular membranes, especially of jejunal and liver epithelial cells. A morphological study of clinical vitamin E deficiency in two children suffering from abetalipoproteinemia and the effect of vitamin E medication on this condition was followed by experiments in which ducklings were raised on a vitamin E-deficient diet. So far these experiments have yielded the following information (Molenaar, Hommes, Braams & Polman, 1968; Molenaar, Vos, Jager & Hommes, 1970; Molenaar, Vos & Hommes, 1972; Molenaar, Vos, Jager & Hommes, 1972; Vos, 1972; Vos, Molenaar, Searle-van Leeuwen & Hommes, 1972, 1973): (1) Cellular membranes of jejunal epithelial cells of patients with abetalipoproteinemia and a resulting vitamin E deficiency, and of ducklings with induced vitamin E deficiency, could not be visualized in the usual way in the electron microscope (Fig. 1); after fixation with osmium tetroxide (which is bound to the double bonds of unsaturated fatty acids) a decrease of positive membrane contrast could be observed in comparison with controls; the same phenomenon was observed in the liver epithelial cells of vitamin E-deficient ducklings. (2) The vitamin E-deficient patients required treatment for 4 months with vitamin E before a completely normal cellular ultrastructure (Fig. 1) could be observed. (3) The mitochondrial membranes, endoplasmic reticular membranes and nuclear outer membranes were morphologically most affected, in contrast with plasma membranes and the membranes of Golgi-lamellae and vesicles; the loss of contrast appeared first and was most marked in the outer mitochondrial membrane (Fig. 2). (4) Electron micrographs of these membrane fractions indicate that the integrity of isolated mitochondrial membranes (especially of outer membranes) decreased or changed under the influence of vitamin E deficiency; in this respect microsomal membranes did not differ from controls. (5) Results of biochemical analysis of isolated mitochondrial inner and outer membranes and of microsomes of liver from vitamin E-deficient ducklings showed that there was specific loss of arachidonic acid; the outer mitochondrial membrane showed a particularly marked decrease in arachidonic acid content (67%) while the decrease for the inner mitochondrial and microsomal membranes was less (43% and 13% respectively); a similar decrease was observed in linoleic acid content of outer mitochondrial membranes (44%), but not in inner mitochondrial and microsomal membranes (<10%) (Table 1). (6) These relative decreases were found to be balanced by increases in palmitoleic and oleic acid contents in outer and inner mitochondrial membranes and by an increase in stearic acid content in microsomal membranes (Table 1). (7) The decrease in membrane-bound polyunsaturated fatty acids is apparently consistent with electron-micrographs showing decreased contrast of certain cellular membranes in tissue from vitamin E-deficient subjects.

These results prompted us to study the membrane proteins, particularly the membrane-bound enzymes. Histochemically, as well as biochemically, a stimulation of glucose-6-phosphatase (*EC* 3.1.3.9), an enzyme of the endoplasmic reticular

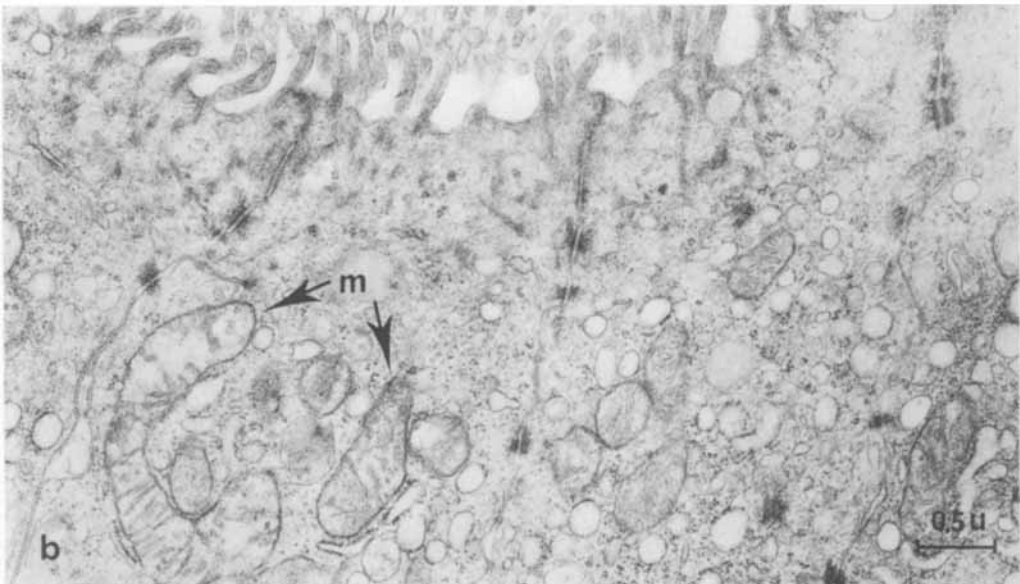
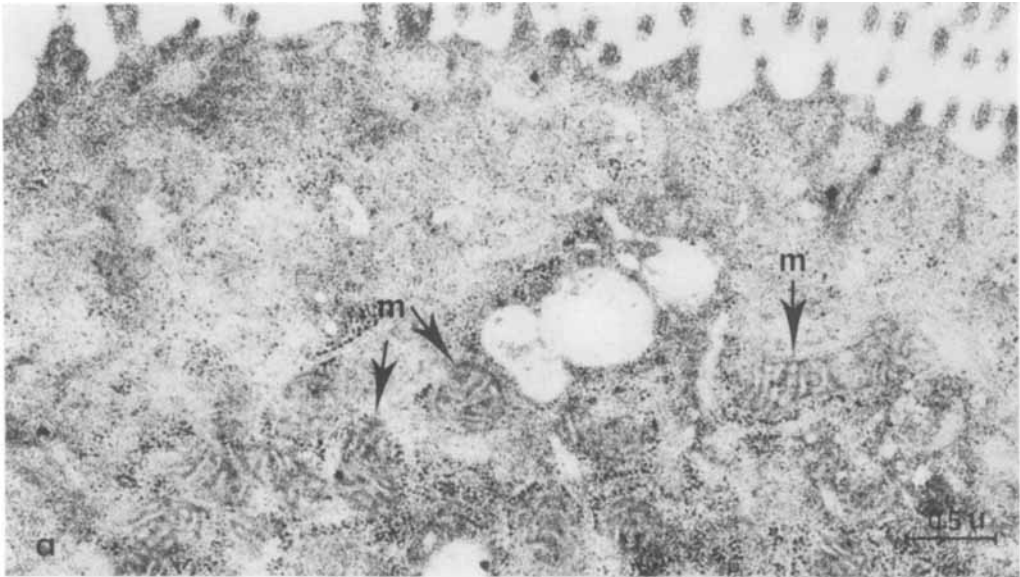


Fig. 1. Jejunal epithelial cells, with parts of microvilli, from a patient with abetalipoproteinemia which resulted in hypovitaminosis E, (a) before and (b) after 4 months treatment with vitamin E. (a) There is a lack of membrane contrast in general. Mitochondria (m) show their matrix, but lack positively contrasted membranes. (b) The cells in general and the mitochondrial membranes in particular have a normal ultrastructural appearance. Membranes are visible in normal contrast. Molenaar *et al.* (1968).



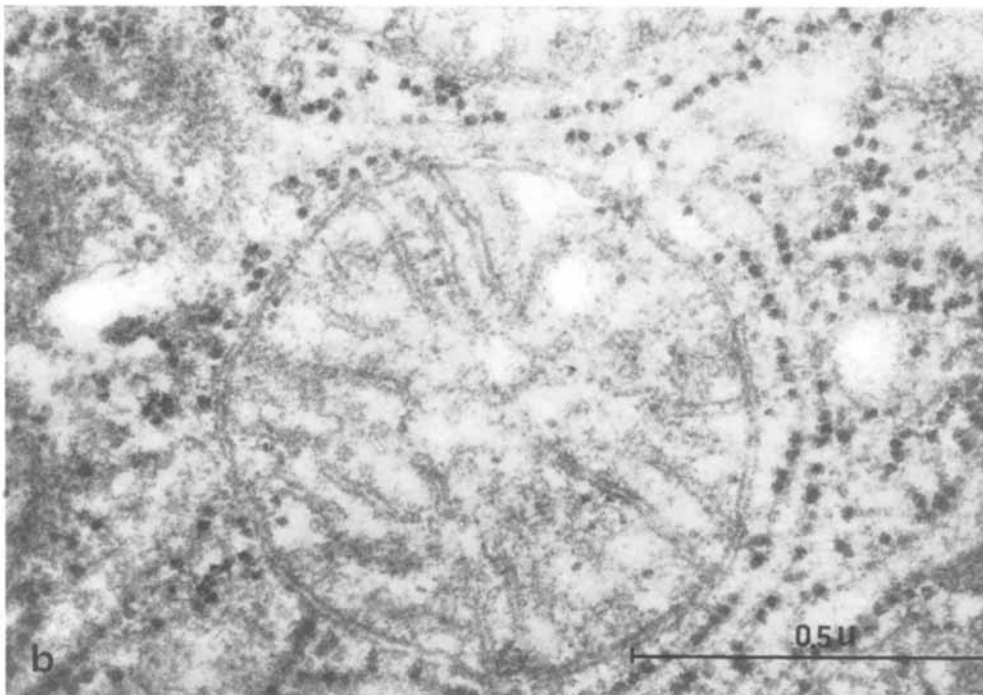
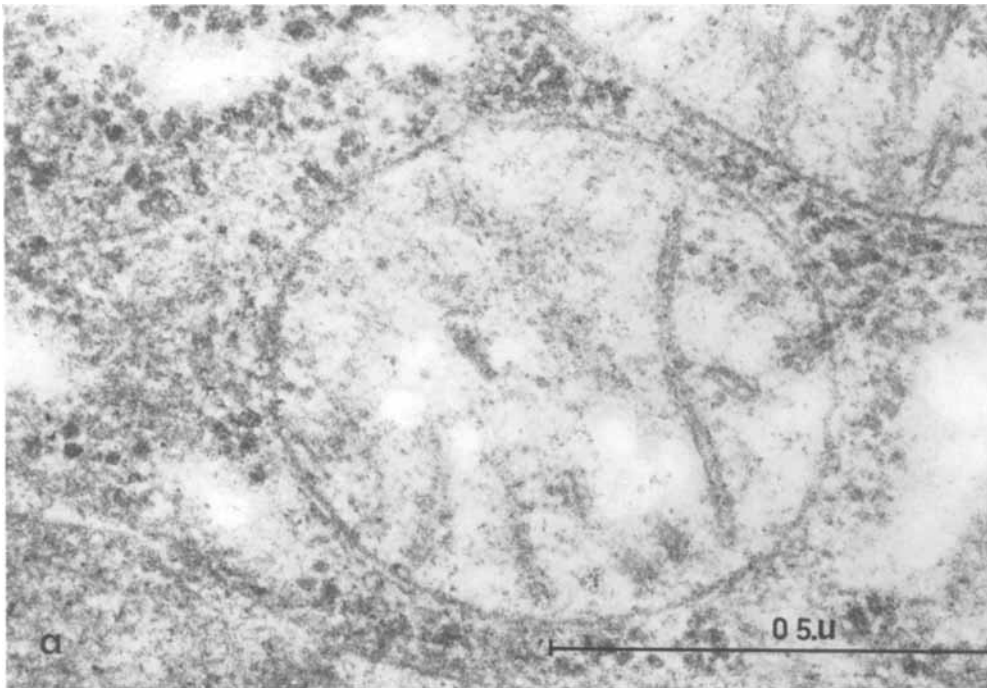


Fig. 2. (a) Jejunal epithelial cell from a vitamin E-deficient duckling. There is a big difference in contrast between the inner and outer mitochondrial membranes. Compare with those in (b). (b) Jejunal epithelial cell from a normal duckling. The electron density of the mitochondrial membranes is similar. Compare with those in (a). Molenaar *et al.* (1970).

Table 1. *Fatty acid composition (expressed as a % of the total fatty acids) of inner and outer mitochondrial membranes and microsomes from livers of vitamin E-deficient (D) and control (C) ducklings*

Fatty acid	Inner mitochondrial membrane*		Outer mitochondrial membrane†		Microsomes‡	
	C	D	C	D	C	D
C 16:0	20.4	24.1	29.6	27.2	25.6	23.9
C 16:1 ω7	1.9	3.3	1.9	3.4	1.6	1.6
C 18:0	25.0	22.1	25.3	26.4	18.2	20.9
C 18:1 ω9	20.9	25.3	21.9	25.2	21.6	20.5
C 18:2 ω6	13.3	12.2	6.8	3.6	4.5	4.7
C 22:0	1.1	0.2	+	+	0.4	1.2
C 20:4 ω6	13.3	7.6	9.4	3.3	16.9	14.7
C 24:0	+	+	0.7	+	1.6	2.2
C 22:6 ω3	1.6	1.0	1.2	2.0	2.0	3.4
Unidentified	2.5	4.0	3.2	8.9	7.6	6.9
Total saturated	46.5	46.4	55.6	53.6	45.8	48.2
C 16:1 ω7 + C 18:1 ω9	22.8	28.8	23.8	28.6	23.2	22.1
C 18:2 ω6 + C 20:4 ω6	26.6	19.8	16.2	6.9	21.4	19.4

\*Average of four determinations, ten control and ten deficient animals.

†Determination of three combined fractions, eight control and eight deficient animals.

‡Average of three determinations, eight control and nine deficient animals.

membrane, and of 5'-nucleotidase (*EC* 3.1.3.5.), an enzyme of the plasma membrane of the bile capillary, has been found in the liver of vitamin E-deficient ducklings (C. E. Hulstaert, unpublished observations).

The clinician who wants to prevent or cure a deficiency must be able to assess the vitamin E status of the patient; in the solution of this problem the membrane-vitamin E concept could be valuable. First, severe vitamin E deficiency in man is rare because the basic requirement for vitamin E can easily be supplied by balanced diets. It has been found that the intake of polyunsaturated fatty acids (PUFA) may increase vitamin E requirement. However, there is no consensus of opinion about the relevance of a fixed critical vitamin E:PUFA ratio (mg D-α-tocopherol/g PUFA) in a diet (Witting, 1972; Jager, 1972) in evaluating the vitamin E adequacy of this diet. Whatever the significance of this ratio, it can be stated that, in general, vegetable oils containing high levels of linoleic acid also contain adequate vitamin E for the basic requirement plus the extra requirement caused by high PUFA intake (Vles & Jager, 1970). Secondly, severe vitamin E deficiency is rare because the vitamin E reserve is probably very large, because vitamin E is stored in the large total membrane surface and also in the vacuoles of fat cells. Thirdly, the values for radioactivity recovered in urine after administration of radioactive vitamin E to rats and rabbits indicate that the metabolic consumption of vitamin E is probably low (Simon, Gross & Milhorat, 1956; Krishnamurthy & Bieri, 1963).

These three factors suggest that severe deficiency in man occurs infrequently. However it is this degree of deficiency which is commonly studied in experimental animals. In man it is subdeficiency particularly which should be detected and investigated because industrial and cooking processes tend to destroy tocopherols in many

foodstuffs (Moore, Sharman & Ward, 1957; Kelleher & Losowsky, 1970; Smith, Kelleher, Losowsky & Morrish, 1971). Jager (1973) has suggested that a high percentage of human beings may be instances of border-line vitamin E deficiency. It is therefore important to know whether a reliable assessment of the vitamin E status is possible.

For the vitamin E status 'uptake' and 'absorption' are important variables. We define uptake as oral intake minus loss in the faeces i.e. the vitamin transported across the plasma membrane of the intestinal epithelial cell. For this process the formation of micelles is important (MacMahon & Neale, 1970; Pearson & Legge, 1972), and this process requires a sufficient secretion of bile (Gallo-Torres, 1970). Therefore an insufficient bile production, resulting from liver disturbances, can lead to a reduced uptake of vitamin E by the intestinal epithelial cell (Kater, Unterecker, Kim & Davidson, 1970; Göransson, Nordén & Åkesson, 1973). A deficient pancreas function, similar to that associated with cystic fibrosis, can have the same result (Darby, Davidson & Desai, 1973).

Absorption of vitamin E is determined by the appearance of the administered compound in the plasma. As 75% of the vitamin E taken up is transported with chylomicrons (Blomstrand & Forsgren, 1968) absorption is dependent on the process of chylomicron synthesis by the endoplasmic reticulum of the intestinal epithelial cells and on the transport of chylomicrons to the blood. Disturbances of the function of the endoplasmic reticulum affecting chylomicron synthesis can, therefore, result in a vitamin E deficiency. This situation is found in abetalipoproteinemia (Molenaar *et al.* 1968) and this disease can be simulated by administration of puromycin to rats. The same effect can be achieved if, by unknown epithelial cell injury, the release of formed chylomicrons is impaired (Ament, Shimoda, Saunders & Rubin, 1972). Whether the same process occurs in sprue (psilosis) is unknown. A vitamin E deficiency resulting from an impairment of chylomicron transport could be found also in stasis of chyle in the intestinal lymphatics (Stoelinga, van Munster & Slooff, 1963; J. H. van Tongeren, personal communication).

A good vitamin E uptake does not necessarily suggest a good absorption (Losowsky, Kelleher, Walker, Davies & Smith, 1972); also a good absorption does not necessarily indicate an adequate tissue-content of vitamin E. It is the localization of vitamin E in the membrane which has to be considered when assessing plasma tocopherol levels. Evidence has been obtained that the body has two pools of the vitamin at its disposal: first a labile one, which is mobilized rapidly (particularly in the blood), and, secondly, a fixed component (Bieri, 1972) which we think most probably corresponds to the membrane-bound vitamin E. However, as we have mentioned, the vitamin exerts its function not in the plasma, but in the cellular membrane, a completely different phase, indicated by the above-mentioned model of Singer & Nicolson (1972). Therefore 'absorption', expressed as  $\mu\text{g}$  tocopherol/100 ml plasma could be at present a misleading variable for assessing the vitamin E status of the tissues (Losowsky *et al.* 1972; Underwood, Denning & Navab, 1972). Considering the rapid exchange of vitamin E between the plasma (Silber *et al.* 1969) and the erythrocyte membrane, the same problem is associated with the haemolysis test on

erythrocytes (Rose & Gyorgy, 1950). Also, it should be stressed that this method uses the cell membrane, shown in our experiments to be the membrane which is least vulnerable to vitamin E deficiency, probably as a result of the relatively high content of saturated fatty acids. Another possibility, scoring myopathy (Jager & Vles, 1970), must be considered to be only relevant in experimental animals. In man no nutritional myopathy caused by vitamin E deficiency has been reported (Jager, 1973).

A more refined approach for assessment of the vitamin E status in man, considering the two pools of the vitamin, should include: (1) an analysis of subcutaneous tissue or a skin biopsy (fixed pool) and (2) determination of plasma levels or erythrocyte haemolysis tests (labile pool); in both instances over a prolonged period of time.

In conclusion, we state that in man and in experimental animals, vitamin E deficiency causes changes in membrane ultrastructure which can be correlated with a change in chemical composition and function, pointing to the membrane as the functional site of vitamin E. Thus, when considering the assessment of vitamin E status, the membrane localization of this vitamin should be considered.

We thank (US) National Academy of Sciences, Washington DC for permission to reproduce Fig. 1.

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